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DEVELOPMENTAL CYCLE OF ELAPHOSTRONGYLUS PANTICOLA, LUBIMOV, 1945.

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V. Ya. PANIN

Among the helminth diseases of maral [an Asiatic red deer] and spotted deer, elaphostrongylosis may be ranked as one of the most important. The pathogenic organism involved is Elaphostrongylus panticola Lubimov, 1945. Elaphostrongylosis of marals and spotted deer is widespread on deer- and stag-breeding farms of Altai and the Maritime Territory According to the data obtained by coprological investigations, the incidence of marals' infection on Verkh-Katunskii and Katon-Karagai sovkhozes (in the Kazakh SSR) was 82.3 and 60.7 % respectively; in spotted deer an incidence of 52.2 % was found (Pryadko, Vysokov and Frolov, 1963).

The pathology of elaphostrongylosis is only imperfectly known, but nevertheless the scant data available (Lyubimov, 1950, 1959; Vsevolodov and Pryadko, 1964) convincingly show that this infection causes grave illness of marals and spotted deer accompanied by damage to the central nervous system and disturbances of physiological functions of the host organism.

Elaphostrongylosis usually causes a general lowering of the animals' productivity (loss of the capacity to produce commercially valuable antlers, or lowering of the antler quality). In some years, however, it assumes the character of enzooties with ensuing mass mortality of animals. For example, in an outbreak of elaphostrongylosis in the winter of 1961/1962 at a maral-breeding sovkhoz in Altai Territory, the mortality of marals due to this infection was 63.6 % of the total death rate in the said period (Pryadko, Vysokov and Frolov, 1963).

The above data are convincing confirmation of the urgent necessity of undertaking the study of elaphostrongylosis in all its aspects in "panty"* deer. At present such studies are being pursued at the Institute of Zoology of the Academy of Sciences in the Kazakh SSR under the direction of S. N. Boev.

Background of biological study of elaphostrongylosis. At present five species of the elaphostrongyl nematodes are known, all of them parasitizing different species of Old and New World deer. The life cycles have been studied in three species. In the Soviet Union only two species have been observed: Elaphostrongylus panticola Lubimov, 1945, obtained from marals (Cervus elaphus sibiricus) and spotted deer (Cervus nippon), and E. rangiferi Mickevitsch [Mitskevich], 1958, from reindeer.

The first investigators to elucidate the developmental cycle of one of the elaphostrongyl species were the Hobmaiers (Hobmaier A. and M., 1934). Having studied the biology of Parelaphostrongylus

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* [Russian "panty" refers to the soft core of young antlers of certain species of European and Oriental deer. Pantocrine, extracted from the "panty, " is extensively used in Russia and the Far East as a medicament and tonic.]

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(=Elaphostrongylus odocoilei), a parasite of black-tail deer (Odocoileus columbianus), these investigators concluded that first-stage larvae do not undergo changes in the external medium but continue their further development in intermediate hosts—the terrestrial mollusks of the Limacidae and Helicidae families. In these intermediate hosts, the invasive stage is reached after two molts, towards the end of the fifth week after infestation. When deer were fed infested mollusks, the Hobmaiers observed first-stage larvae in the feces 4 to 5 months after the infection.

In studies of the development and the biology of the helminth E. panticola, M. P. Lyubimov (1959) established the role of intermediate hosts—the terrestrial mollusks Succinea putris and Bradybaena fruticum. By experimentally infecting mollusks, the development periods of larvae in the intermediate host up to the invasive stage were ascertained. It transpired that the rate of larval development is not uniform, and depends upon the species of the mollusk involved. Thus, in S. putris invasive larvae develop 30 days after the infection, in B. fruticum, 44 to 50 days after.

V. Yu. Mitskevich (1958) has described a new species of elaphostrongyl, Elaphostrongylus rangiferi, obtained from reindeer, and conducted investigations on its developmental cycle, with the following results: first-stage larvae are capable of surviving in a free state up to two years, but do not grow or change morphologically under such conditions. When larvae are fed to experimental animals no infection develops, since for their further development an intermediate host is required. Intermediate hosts are various species of terrestrial and freshwater mollusks. In mollusks, larvae undergo two molts and within 24 to 35 days after infection reach the invasive stage. The development to sexual maturity of E. rangiferi in the final host lasts three or four months.

It thus appears that the developmental cycles of the above three species follow the same pattern. The variations found in each case are insignificant and do not affect the general course of this type of development, inasmuch as they only concern differences in species of intermediate hosts or relate to the duration of the stages of the parasite's development in the intermediate and definitive hosts.

The present investigation

This report is devoted to a more detailed study of the developmental cycle of Elaphostrongylus panticola, and touches upon the following questions: 1) methods of multiplication, 2) the behavior of first-stage larvae, 3) types and species of intermediate host and 4) character of development in the intermediate and definitive hosts.

The research described here was conducted in 1961 to 1963 on Katon-Karagai and Verkh-Katunskii maral sovkhozes in the East Kazakhstan Region (southern Altai)*.

* The author expresses his gratitude to laboratory assistants S. N. Vysokov, O. I. Rusikova, R. Izatulaeva, and M. N. Olenchenko, for their help in the collection and dissection of mollusks.

Methods of multiplication. Special experiments on female elaphostrongyls in artificial media were not conducted. My conclusions on the manner of reproduction are based on data obtained by microscopic examination of organs (brain, lungs) of dissected marals. On examination of smears from membranes of the brain and spinal cord, as well as microscopic investigation of lung tissue, eggs and first-stage larvae of elaphostrongyls were detected. In scrapings from the trachea only first-stage larvae were found; eggs were not present in this tissue. Histological examination of brain and lungs of marals (Vsevolodov and Pryadko, this collection) revealed masses of eggs in various stages of development, and elaphostrongyl larvae present in the vascular parts of the dura and pia mater of the CNS and, in smaller quantities, in the lung.

As the data of B. P. Vsevolodov and E. I. Pryadko and my own observations show, elaphostrongyl reproduction is exclusively oviparous. Since sexually mature parasites are absent from the lungs, it may be assumed that oviposition and mass emergence of larvae from the eggs occur in the CNS and in other organs which elaphostrongyls parasitize. On hatching, the larvae and a portion of unripe eggs are carried in the bloodstream from locales of sexually mature parasites to the lungs. The further fate of elaphostrongyl eggs which find their way into the lungs remains an open question. Inasmuch as no eggs were detected in the trachea, it appears to me that marals cough up and swallow only first-stage larvae with the bronchial mucus. These larvae are subsequently voided with the feces.

Structure of first-stage larvae (Figure 1). First-stage larvae, voided in the marals' feces, are transparent. The body is covered with a smooth cuticle. The foregut, not always discernible, is characterized by the presence of two sections. One is more pronounced and is situated in the posterior part, where it borders with the hindgut; the other part is short, and is 0.070 to 0.073 mm distant from the anterior end. The hindgut is granular along its entire length but only faintly so, and this makes the boundary between the fore- and hindgut difficult to discern. The tail end is characterized by the presence of a dorsal spikelet, located a little before the end of the body.

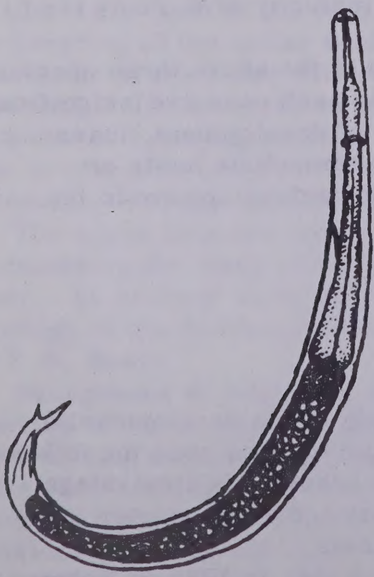

FIGURE 1. First-stage larva (× 400)

The dimensions of these larvae (based here on the measurements taken of 100 larvae) are : length of body — 0.352 to 0.425 mm; maximum width — 0.019 to 0.022 mm; width of head end — 0.004 to 0.006 mm; width of body in the region of the anus — 0.011 to 0.014 mm; foregut — 0.165 to 0.190 mm; the excretory aperture is 0.101 to 0.117 mm distant from the head end; the neural ring is 0.031 mm distant from the head end; the rudimentary sprout of of the sex organ is 0.011 mm long and 0.006 mm wide; the anus is 0.034 to 0.042 mm distant from the head end; the length of the tail (from the base to the pointed tip) is 0.009 to 0.011 mm; the length of the dorsal spikelet is 0.0028 mm.

Survival of first-stage larvae in external medium. Elaphostrongyl larvae voided with marals' feces can survive in the external medium for a long time. The resistance to drying of these first-stage larvae, as well as their resistance to solar radiation and high and low temperatures, was studied by M. P. Lyubimov (1959), and therefore will not be dealt with here. In Lyubimov's experiments elaphostrongyl larvae survived for almost a year.

My own observations have confirmed that larvae can survive even longer under natural conditions on marals' pastures. In one of the parks in Verkh-Katunskii sovkhoz marals had not been kept for over 20 years. In 1961 female adult and yearling marals were brought into that park from other parks, and were kept there during the whole summer. In autumn the animals were transferred to winter quarters, and in summer of the next year let out into other parks. For the space of two years (1962—1963) no marals were pastured on the park in question, and consequently no accumulation of invasive elements could have taken place during this period on the pastures. Only larvae voided with marals' feces during the latter's stay on the pasture during 1961 could have remained. In 1962 feces collected from the pastures were examined by Berman's method. In each sample a large number of elaphostrongyl larvae was detected. However, elaphostrongyl larvae were no longer present in the feces collected from the pastures in the summer of 1963. These findings justify the conclusion that in natural conditions first-stage larvae remain viable for a period of from one to one and a half years.

The great durability of first-stage larvae is further proved by an experiment keeping them in water. Thus, when larvae were kept in water (which was changed once every ten days) at a temperature of 18°—20°C, 20% of the larvae remained alive at the end of 4 months. The observations were not pursued further. When larva just voided from maral organisms were compared with those kept four months in water, no differences were observed in their dimensions or structure. Consequently, first-stage larvae do not grow or change morphologically in the external medium.

It was of interest to study whether the biological activity of first-stage larvae was altered during a long period of exposure to the external medium. In other words, do first-stage larvae retain the capacity to infest the intermediate host and to continue their developmental cycle within the body of the intermediate host to the invasive stage? To answer this question experiments were carried out on artificial infection of intermediate hosts by exposure to elaphostrongyl larvae which had been in the external medium for prolonged periods. Experimental infection of mollusks has shown that elaphostrongyl larvae, after remaining in feces for about four months, do not lose their capacity to penetrate into intermediate hosts and to develop normally there to the invasive stage. In these experiments the larvae-containing feces were kept in small bags in a garret from February to May. Under these conditions several factors (solar radiation, dampness, low temperature) which might affect the biological activity of larvae were not as marked as in natural conditions. However larvae in mollusks infected from feces left on the pastures for about a year did not lose the capacity of further development in the intermediate hosts. In this case, however, development periods were somewhat longer; this will be discussed below.

Movements of larvae on the substrate. The ability of larvae to migrate vertically was studied in the following manner. Sterile soil was placed in a crystallizing basin or in a large pot and barley was sown in it. When the barley grew to the height of 15—20 cm, the vessel's surface was divided into sections. Several fecal pellets from marals artificially infected with elaphostrongyls were placed on each section. Then the feces and the surface layer of the soil were moistened with tap water. At given intervals barley stems were harvested and cut up (the upper, middle, and lower portions were kept separate), pulverized, and examined by Berman's method. These experiments established that after a few hours elaphostrongyl larvae move out of the feces and begin migrating onto the plants. After 24 hours, 37 larvae were found on the barley stems, of which 25 were on the lower portion of the stem (67.5%), 12 on the middle portion (32.5%), and none on the upper portion. After 48 hours, 46% of the larvae were on the lower portion, 39.5% on the middle portion, and 14.5% on the upper portion of the stem.

The speed of movement depends to a great extent on the dampness of the soil, feces and plants. Plentiful moisture coincides with an increase in the number of larvae on the middle and upper portions of the stems. In these experiments, the average numbers of larvae were: on the upper portion of the stems, 28; on the middle, 35; and on the lower, 50. Under conditions of low humidity most of the larvae remain on the lower stem portions; on the middle portions only solitary larvae are observed, while larvae were totally absent from the upper portions of the stems.

The study of horizontal larval migration was conducted in Petri dishes, the bottom surfaces of which were divided into small squares by means of a glass cutter. The dishes were filled with a thin layer of gelatine, whose surface was moistened with water. Then on one of the squares a drop of water containing elaphostrongyl larvae was placed. Larval movements were observed and followed through a binocular microscope. These experiments lead to the conclusion that elaphostrongyl larvae are capable of movement on a horizontal plane as well, although the speed of these movements is comparatively low. In 15 to 20 minutes larvae are able to move only 1 cm from the original position.

From the above experiments, it appears that first-stage larvae are capable of both vertical and horizontal movements.

Intermediate hosts of E. panticola. The general outlines of the life cycle of E. panticola has already been described (Lyubimov, 1959). Elaphostrongyl development is basically similar to that of the other protostrongylids, i.e., it involves association with an intermediate host, in this case, terrestrial mollusks.

In order to determine which species of mollusks can serve as intermediate hosts of E. panticola at the deer-breeding farms in the Altai Territory, mollusks found in the pastures of marals and spotted deer were dissected and subjected to artificial infection with elaphostrongyl larvae. Althogether 8947 mollusks belonging to 20 species were examined. Larvae of elaphostrongyls were detected in 15 species (see Table).

Those mollusk species found to be infected with elaphostrongyl larvae under natural conditions proved to be susceptible as well to artificial infection in the laboratory.

Apart from terrestrial mollusks, freshwater mollusks may serve as intermediate hosts to E. panticola. In experimental conditions, Radix ovata was easily infected and the elaphostrongyl larvae developed normally in these mollusks.

The role played by particular species of mollusks in the life cycle of E. panticola is discussed in detail in another paper of mine so this matter will not be dealt with here.

Development in intermediate host. Mollusks collected from parts of pastures not used for marals' pasturage (outside the parks) were used in the experiments of artificial infection by elaphostrongyl larvae. From these plots, 2543 mollusks were examined, yet in no case were nematodes detected. Only larval trematodes and cestodes were found. These mollusks, then, were sterile with respect to elaphostrongyl larvae, and could be used in the experiments without any risk.

TABLE. Terrestrial mollusks on pastures of marals and spotted deer examined for E. panticola larvae

Species	Total Number	Infected	
		number	%
Agriolimax agrestis (L.)	288	17	5.9
Cochlicopa lubrica (Müll.)	384	5	1.3
C. lubricella (Menke)	122	7	5.7
C. pseudonitens Ulvalieva	269	17	6.3
Columella columella (Drap.)	427	—	—
Euconulus fulvus (Müll.)	472	10	2.1
Bradybaena fruticum (Müll.)	552	47	8.5
Discus ruderatus (Müll.)	494	16	3.2
Perforatella bicallosa (Pfr.)	119	7	5.9
Perpolita petronella (Pfr.)	709	1	0.1
Pupilla muscorum (L.)	376	—	—
Succinea altaica (Mart.)	930	74	8.0
S. granulosa (Lindh.)	301	11	3.7
Vallonia costata (Müll.)	343	—	—
V. tenuilabris (Al. Br.)	285	—	—
Vertigo substriata (Jeffr.)	454	—	—
Vitrina rugulosa (Mart.)	730	8	1.1
Zenobiella nordenskioldi (West.)	993	236	23.8
Z. aculeata Uvalieva	352	38	10.8
Zonitoides nitidus (Müll.)	347	19	5.5
	8947	523	5.7

The experimental mollusks were kept in large plywood boxes with the bottom covered with turf. The upper openings of the boxes had gauze stretched over them. Leaves of juicy grasses, carrots and potatoes were placed on the turf as food. The food was changed daily, and the turf every 10 days. The surface of the turf and plants was periodically moistened with water. Each box of 50×50×40 cm was filled with 100 to 120 mollusks. The slugs were kept in 1-liter glass jars, with a layer of moist blotting paper and juicy leaves placed at the bottom. The paper was replaced daily. From 5 to 7 slugs were introduced into each jar.

Infection of mollusks was carried out in Petri dishes, with 1 — 2 mm water containing larvae. Collection of larvae for the experiments from marals' feces was carried out by Berman's method. Infection on each mollusk was carried out in separate dishes. After 2 to 3 hours the mollusks were taken out of the dishes and placed in the boxes, where they were kept until the end of the experiment. In some cases, the mollusks were infected by introducing larvae into the orifice of the shell with a pipette.

The mollusks were dissected at various intervals. After infection: 1, 5, and 10 hours; then after 2, 5, 10, 15, 20, 25 days, and so on. For examination, most of the species of experimental mollusks were crushed; only in the case of Bradybaena fruticum, which has very elastic and strongly pigmented tissues, was it necessary to boil the tissues in artificial gastric juice. It was not difficult to detect elaphostrongyl larvae in the mollusk's foot; even in the first hours after penetration larvae were easily spotted under 16× or 24× magnification in the binocular dissecting microscope. For early, successful detection of larvae, an appropriate thickness of the preparation is required. Close examination of the transparent, motile larvae is facilitated in the thinnest possible preparations. Later, larvae become readily detectable in preparations of any thickness, since several days after infection the gut becomes dark colored (Figure 2).

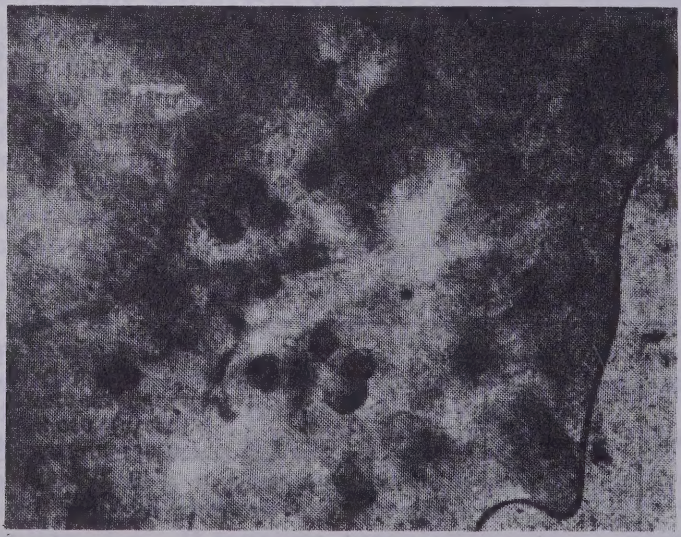

FIGURE 2. Elaphostrongyl larvae in mollusk's foot (× 40)

No special studies to determine the manner of larval penetration into the intermediate host were made. However, observations made a few hours after infection indicate that the larvae actively invade directly the tissues of the mollusk's foot. Thus, as early as 3 hours after exposure, larvae were detected in the foot. Had the larvae been passively taken up by the mollusk in the food, they would have been detected during the first hours' examinations in the mollusks' intestine or in intestinal contents. However, no larvae were found in the intestine and other organs, even after longer periods. Additional proof of the active manner of penetration was provided when mollusks were infected by introducing larvae directly into the orifice of the shell. In such cases the possibility of larvae being passively introduced with food was excluded, yet invasion of elaphostrongyl larvae into mollusks was established on dissection.

It was found that during the first two or three days after infestation into the mollusk's foot, larvae still retain mobility. Later movements slow down and larvae curl up into a spiral; a dark brown pigment accumulates around the larval body, apparently a result of the host organism's reaction to the presence of the parasite. At this stage, no morphological changes or growth of the parasite have occurred.

Subsequently larvae begin to grow in size and undergo morphological changes. I regard this state of the larvae as the beginning of transition to the second larval stage and as preparation for the first molt. On the 5th day after infection all the larvae in the mollusk's foot are immotile, curled up in spirals. The bodies of some larvae begin to be covered by fine particles, dark in color.

The dimensions of such larvae are: body length, 0.397—0.412 mm; width, 0.035—0.041 mm; length of gullet, 0.166—0.193 mm; excretory aperture 0.12—0.128 mm from the head end; anus 0.041 mm from the tail end. The dorsal spikelet is well formed. On being extracted from the mollusk's foot and immersed in water, these larvae are still able to move, although their movements are not as vigorous as those of first-stage larvae.

On the 10th day the body and gut of most of the larvae are pigmented. The dimensions of larvae have markedly increased: body length, 0.496—0.697 mm; width, 0.041—0.054 mm; length of gullet, 0.192—0.217 mm; anus 0.051 mm from the tail end.

On the 15th day all larvae's bodies are covered with formations resembling fat droplets, distributed almost uniformly over the entire length of the body. The gut is filled with black granules. The boundaries of the gullet are hardly discernible. In different parts of the body, especially at the head end, cuticular formations are observed resembling the lobes found in some sexually mature nematodes. These formations result from exfoliation of the old cuticle, and are evidence that the larvae are entering into the first molt.

At this stage, larval dimensions are: length of body, 0.789—0.819 mm; width, 0.057 mm; length of gullet, 0.224—0.256 mm; excretory aperture 0.131—0.139 mm distant from the head end.

On the 18th to 20th day the cuticle has become markedly detached from the body, forming a well discerned sheath. This can be seen particularly clearly at the head and tail ends.

The distances between the head and tail ends of the sheath and the larva's body are extremely variable, as the larva is capable of moving inside the sheath and often changes its position. The formation of the sheath around the larva's body is evidence of the termination of first molt, of the total completion of the second stage of development, and the transition to a new stage.

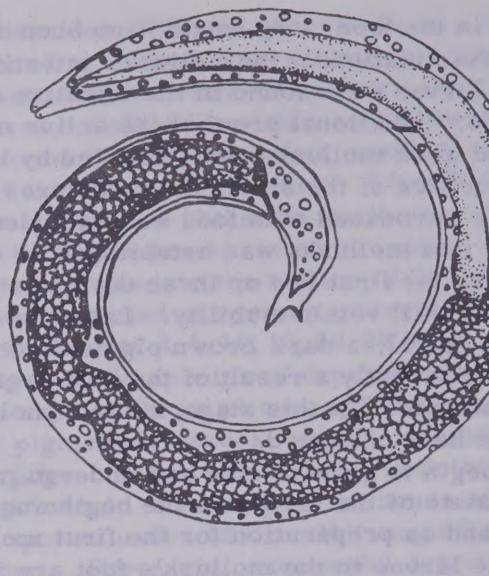

FIGURE 3. Second-stage larva (×200)

The second-stage larva (Figure 3) is 0.793—0.856 mm long and 0.041—0.044 mm wide. Its body is feebly pigmented, but the dark granularity in the intestine is still retained; thus the outlines of the gullet and its boundary with the gut stand out clearly. The dorsal spikelet at the larva's tail end is hardly discernible whereas it is distinctly seen on the sheath. The gullet is 0.249—0.257 mm long; the excretory aperture is 0.134—0.140 mm distant from the head end, and the anus is 0.056 mm from the tail end. The transparent, smooth sheath is 0.808—0.879 mm long and, at most, 0.048—0.054 mm wide. Such larvae, on being extracted from the mollusk's foot, are only slightly motile, but on being squeezed out of the sheath their movements become more vigorous.

Further changes during development include increased body dimensions and decrease in pigmentation of the body and gut. The larva begins the second molt by the 25th day. It continues to remain slightly motile. In some parts of its body the exfoliation of a second sheath may be observed. The body and gut grow more transparent and become pale yellow in color. Such larvae are 0.818—0.958 mm long; maximum width is 0.038—0.044 mm, and the gullet is 0.252—0.308 mm long. The excretory aperture is 0.137 mm from the head end and the anus is 0.056 mm from the tip of the tail.

The larva completes the second molt by the 30th to 32nd day, and passes into the third (invasive) stage. The two sheaths are clearly visible only when pressure is applied to the larva's body (Figure 4). The new cuticle of the invasive stage, like that formed after the first molt, is smooth. The invasive larva's body is pale yellow in color and devoid of granularity, the gullet and gut are clearly visible. The fully formed gullet is characterized by two widened portions. At the head end there are two pairs of small nipple-shaped projections. The dorsal spikelet at the base of the tail is absent (Figure 5).

The dimensions of the invasive larvae are: length of body, 0.910—1.11 mm; maximum width, 0.042—0.050 mm; length of the gullet, 0.308—0.336 mm; central portion of the first widened section of the gullet, 0.098—0.103 mm distant from the head end; the pinlike second widening is at the transition boundary of the gullet into the gut. The chitinized pharynx is 0.017 mm long and 0.005 mm wide. The excretory pore opens at a distance of 0.134 to 0.137 mm from the head end. The sex rudiment, 0.028—0.031 mm long and 0.008 mm wide, is located at a distance of 0.504—0.528 mm from the head end on the ventral side of the body. The anus is 0.053—0.061 mm distant from the tail end.

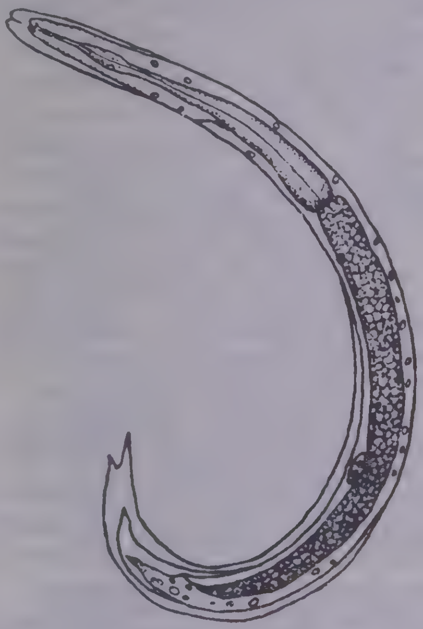

FIGURE 4. Third-stage larva in the process of molting (× 165)

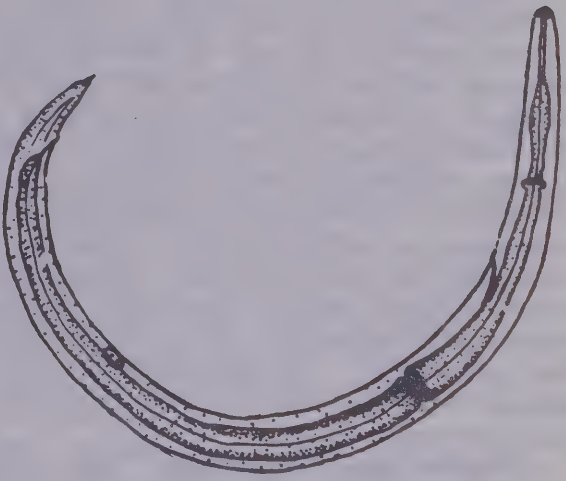

FIGURE 5. Invasive larva (× 150)

The above larvae were found to be capable of infecting marals. The implication of this is that a month after penetrating into intermediate hosts and completing two molts, larvae complete their development and become invasive. If they remain in the intermediate host's organism for a longer period, larvae are capable of somewhat enlarging their dimensions. However, they evidently undergo no further changes of any qualitative significance, inasmuch as their morphological structure and the capacity to infect definitive hosts remain unchanged.

On the 60th day the length of the larva's body reaches 1.28 mm and on the 80th day, 1.29 mm, thereafter remaining almost unchanged. After ten months' sojourn in the intermediate host, the larva's body is 1.35 mm long.

The above data relating to periods of elaphostrongyl development in the organism of an intermediate host apply to only one mollusk species, *Zenobiella nordenskioldi*. Development periods of larva in mollusks of other species differ from the above, tending to be longer.

Thus, in Vitrina rugulosa and Euconulus fulvus the first larval molt is observed on the 23rd or 24th day, and the invasive stage is reached 36 — 38 days after infection. In freshwater mollusks larvae develop even more slowly. The first molt of elaphostrongyl larvae in Radix ovata is observed after 28 — 30 days, and the full development is completed by the 45th day. The rate of larval development in B. fruticum and in species of Succinea is similar to that in Z. nordenskioldi. This is confirmed by the experimental infection of marals by larvae after 32 days' incubation in these mollusks.

It should be mentioned that the period required for elaphostrongyl development in an intermediate host depends upon the biological condition of first-stage larvae. Larvae which had remained for four months (February to May) in feces in the garret reached the invasive stage in Z. nordenskioldi on the 35th or 36th day. Larvae which had remained for a year in feces in pastures take even longer to develop and (in Z. nordenskioldi) reach invasive stage by the 42nd day.

To determine whether invasive larvae actively leave the intermediate host's body and emerge into the external medium, plants and soil from terraria and slime from the mollusks' foot were examined after Berman's method. In addition, experiments were made. After larvae had reached the invasive stage, host mollusks were kept for a month in Petri dishes or in wide-mouth jars. Before this a few mollusks were dissected in order to determine the intensity of infestation. Washes of the cups and jars were examined periodically under a magnifying glass. Slime excreted by the mollusks was also examined. Elaphostrongyl larvae were not detected in any of these cases. Dissection of experimental mollusks at the end of the experiment showed that the average intensity of infestation remained at the same level. The conclusion is, then, that elaphostrongyl invasive larvae do not actively leave the intermediate host's body to emerge into the external medium.

Invasive larvae's life span. In a mollusk's foot, invasive larvae show no signs of movement and lie curled up in a spiral. When squeezed out of the foot of a mollusk or when carefully extracted with a needle, they gradually acquire mobility. Then their movements become more vigorous, but in a few hours they quieten down, curl up into a spiral and again become quiescent. They can apparently remain in such a state for a long time. After 3 days in water, about 17% of the invasive larvae still retain mobility; the rest perish. In the course of these studies no case of larval emergence from the covering sheath was observed.

No conclusive data are available on the life span of invasive larvae inside the intermediate hosts. M.P. Lyubimov (1959) found that elaphostrongyl invasive larvae remained alive after a period of 8 to 15 months spent in the foot of B. fruticum.

Our experiments to determine the invasive larvae's life span in the intermediate hosts were pursued in two directions: by observing experimentally infected mollusks, and by periodic examinations of mollusks on pastures not used for the two preceding years for maral pasture. In both cases only one species of mollusk, Z. nordenskioldi, was examined.

The experimentally infected mollusks were kept in conditions of Altai summer for three months and then brought to Alma-Ata. While a portion of the mollusks were used in other experiments on infecting definitive hosts, the remainder (50) were kept throughout the following autumn, winter and summer in out-of-doors' conditions. Only 10 mollusks survived the winter. Elaphostrongyl invasive larvae were detected in the feet of mollusks which had perished. A year after the start of the experiments observations had to be discontinued since all the mollusks had died. Along with live invasive larvae, larvae without any signs of mobility were found in the feet of such mollusks. Small capsules of dark brown color, enclosing larvae or fragments of their bodies, were observed as well.

Examination of mollusks in an experimental park gave the following results. During the first year after discontinuance of marals' pasturage in the park, the extent of incidence of invasion of mollusks by elaphostrongyl larvae was 92.7% (127 out of 137). The following year, three years after mollusks were infected with elaphostrongyl larvae, examination of 200 Z.nordenskioldi mollusks gave negative results. Capsules were found in mollusks' feet, such as those seen in the experimental mollusks; however, these capsules did not contain any larvae or fragments. It appears that invasive elaphostrongyl larvae perish after two years' existence in the intermediate host. It must be remembered, however, that considerable numbers of infested mollusks must have died during this interval. In all likelihood these two factors (death of larvae in the mollusks and death of mollusks themselves) operated simultaneously, resulting in the sharp drop of elaphostrongyl invasion on the pasture.

This research does not provide a complete solution to the question of the length of elaphostrongyl larvae's survival in the intermediate host. For this, supplementary observations are required, which would be complex and laborious due to various factors (the difficulty of keeping mollusks for long periods, control checks of larvae's invasive capacity, involving especially laborious experiments, etc.).

Development in definitive host. For the determination of development periods of E.panticola in definitive hosts experimental animals were infected with invasive larvae taken from experimentally infected S.altaica, Z.nordenskioldi and B.fruticum mollusks. Two 4-year-old marals (a male and a female), two sheep (one year old), and a 3-month-old roe deer were the subjects. Before the start of the experiment the older animals were kept (from one to four years) in open-air cages at the experimental station of the Institute of Zoology of the Academy of Sciences of the Kazakh Republic (AN KazSSR). Consequently, any contact with invasive elements had been excluded. For four days preceding the beginning of the experiment daily coprological examinations of all the animals by Berman's method gave negative results.

The feet of infested mollusks were carefully crushed between glass slides and the number of larvae in each mollusk was counted. The excised mollusks' feet were then fed to experimental animals together with oats or bread. The dose of larvae was: 500 to marals, 300 to roe deer, and over 700 to sheep.

For 10 days after the start of the experiment, the feces were examined daily, after Berman's method. Later examinations were made every three or four days.

Four months after the experiment began the female maral showed signs of nervous depression, refused to take food, and her movements became sluggish. Examination had not revealed any larvae in her feces. After four days, when the general state of the animal improved, elaphostrongyl larvae appeared in her feces. In the male maral, voiding of larvae began six days later. Thereafter the numbers of voided larvae gradually increased. Thus, on the first appearance of larvae in the feces, one sample (20 g) contained only four larvae, a similar sample ten days later contained 45, after 20 days — 247, after 30 days — 782, after 40 days — 1260, and after 50 days — over 1500 larvae. Beginning with the 55th day after the first appearance of larvae (six months after the infection) the concentration of larvae in feces remained almost static — about 2000 in each sample.

Coprological examinations of sheep gave negative results. Four months after their infection, the sheep were dissected. Examination of the brain and spinal cord, lungs, scrapings of trachea and bronchiae, skeletal muscles, liver and spleen did not reveal elaphostrongyls.

The roe deer was dissected 70 days after infection; no parasites were detected.

Thus only marals proved susceptible to elaphostrongyls in these experiments. The immunity of sheep to elaphostrongylosis is also confirmed by the examination of sheep grazing on infected maral pastures.

The developmental cycle of E. panticola may be described as follows. Sexually mature females of the species lay eggs in specific locales — brain, spinal cord, muscular connective tissue. Here the eggs develop and the larvae hatch. On emerging from the eggs larvae are carried in the bloodstream to the heart and via the pulmonary artery penetrate into the lungs. The larvae are coughed up to the mouth in bronchial mucus and swallowed. Finally, larvae are voided in the feces into the external medium.

First-stage larvae emerging from the definitive host are very resistant to various factors of the external medium, and are capable of retaining biological activity up to 1 — 1½ years. In the external medium larvae neither grow nor undergo morphological changes.

A necessary requirement for continuation of the biological cycle is larval migration from the feces to the surrounding vegetation or to the surface of the soil, and eventual penetration into an intermediate host. Invasion of the intermediate hosts, terrestrial mollusks, occurs when mollusks are most active, crawling on grass or soil on rainy or cloudy days. The first-stage larvae actively penetrate into the foot of a mollusk, where they undergo further development. Larvae begin to feed in the intermediate host, storing up nourishment, as evidenced by the dark granular coloration of the gut. In the intermediate host, larvae undergo two molts and morphological changes involving the formation of sheaths and reconstruction of the gullet.

The first molt in the obligate intermediate host takes place 18 to 20 days after invasion. The old cuticle exfoliates and is replaced by a new one. The covering sheath formed from the old cuticle during the first molt is not cast off. Apart from increases in body dimensions, there are also changes in the morphological structure: the formation of the sheath, intense pigmentation of the body and intestinal components and disappearance of the dorsal spikelet at the tail end of the larva's body. This completes the second larval stage.

In the course of further development the intestinal granularity disappears. The larva's body becomes straw colored. The second molt ensues on the 30th to 32nd day, and the larva passes into the third invasive stage. The cuticle is not cast off after the second molt, and thus invasive larvae in the mollusks' foot are covered by two sheaths, which cling tightly to one another and to the larva's body. Occasionally the sheaths are discernible at the head end.

Invasive larvae do not spontaneously emerge from mollusks, remaining in the intermediate host for long periods, up to two years and possibly even longer. Definitive hosts are infected by swallowing infested mollusks in grass.

The course of invasive larvae's migration within the definitive host has not been defined. The development of E. panticola in the maral from infection to excretion of larvae in feces takes four months. Mass voiding of larvae is observed five or six months after infection. The duration of sexually mature elaphostrongyls' life in the definitive host has not been determined. We observed excretion of larvae by marals even ten months after infection.

Conclusions

1. Elaphostrongylus panticola is oviparous, with larvae hatching from the eggs in the locales of the sexually mature parasites. Via the blood circulation, the larvae reach the lungs. Larvae are coughed up and swallowed in bronchial mucus and ultimately are voided from the digestive tract in the feces.

2. Larvae voided in feces possess the capacity to perform vertical and horizontal migrations. The most intensive larval migrations occur under conditions of maximum humidity (on rainy, dewy days).

3. Both terrestrial and freshwater mollusks can serve as intermediate hosts for E. panticola. In conditions prevailing in Altai Territory, 15 species of terrestrial mollusks were found to serve as intermediate hosts for E. panticola: Agriolimax agrestis, Cochlicopa lubrica, C. lubricella, C. pseudonitens, Euconulus fulvus, Bradybaena fruticum, Discus ruderatus, Perforatella bicallosa, Perpolita petronella, Succinea altaica, S. granulosa, Vitrina rugulosa, Zenobiella nordenskioldi, Z. aculeata, Zonitoides nitidus. The freshwater mollusk Radix ovata was also susceptible to larvae of elaphostrongyls.

4. The obligate intermediate hosts are Z. nordenskioldi, S. altaica, and B. fruticum.

5. Elaphostrongyl larvae go through two molts in the intermediate host. The first occurs 18 to 20 days, the second 30 to 32 days after the penetration of larvae.

6. Larval development in the body of the intermediate host to the invasive stage lasts 30 to 45 days, depending upon the mollusk species and on the biological condition of the larvae.

7. Invasive larvae do not actively emerge from the mollusk into the external medium. According to our observations, larvae can survive in the intermediate host's organism for about two years. Consequently, definitiv hosts contract infection by swallowing infested mollusks with grass feeds.

8. The development in definitive hosts (from infecting to initial appearance of larvae in feces) lasts four months. Mass excretion of larva occurs five to six months after infection.

9. Completion of the entire developmental cycle in E panticola requires five to seven months.

10. E. panticola probably is a specific parasite of marals and spotted deer. In other species of animals (sheep, goats, cattle, horses) pastured together with marals, these parasites have not been detected. Attempts to infect sheep artificially failed.

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